HU:MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSL
MO:MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL

HU:GGPAPPPAPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG
MO:GGPAPPPAPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG

HU:ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS
MO:ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU:YGHTPSHHAAQFPNHSFKEEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG
MO:YGHTPSHHAAQFPNHSFKEEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG

HU:SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE
MO:SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE

HU:GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS
MO:GQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS

HU:ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR
MO:ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR
MO:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR

HU:WPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
MO:WHSCQKKFARSDELVRHHNMHQRNMTKLHVAL

FIG. 1

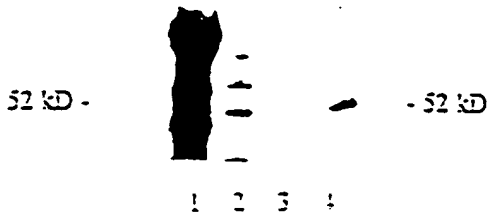


FIG. 2

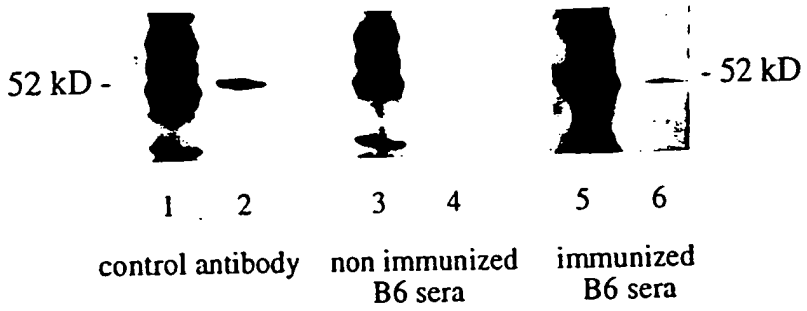

52 kD -
- 52 kD
1 2
3 4
5 6
control antibody
non immunized B6 sera
immunized B6 sera

FIG. 3

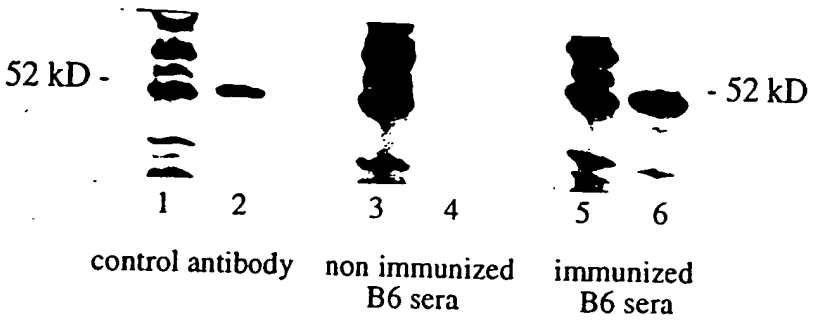

52 kD -
- 52 kD
1
2
3
4
5
6
control antibody
non immunized B6 sera
immunized B6 sera

FIG. 4

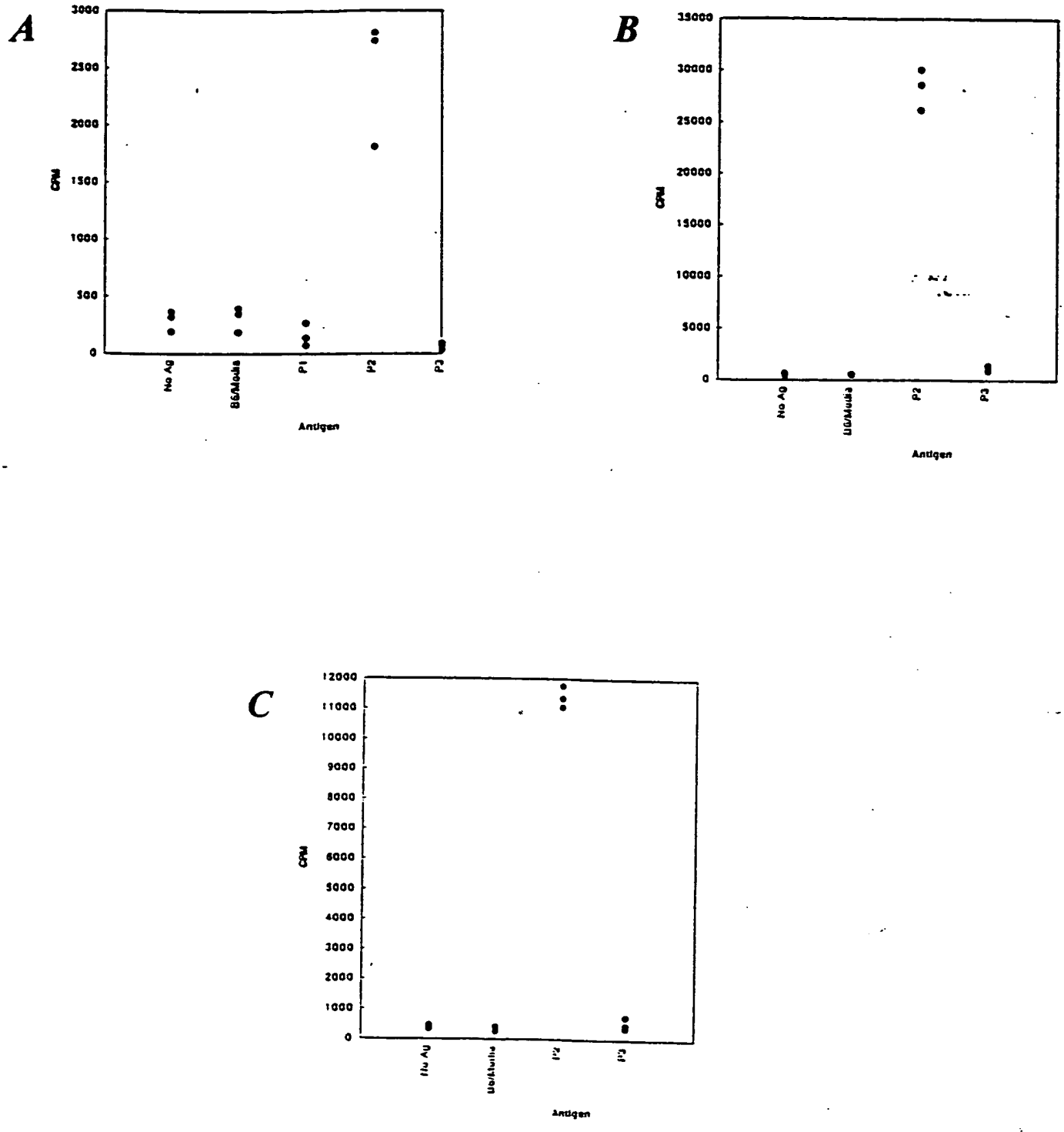
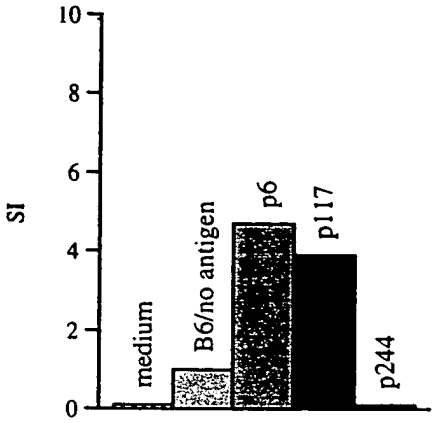

A
3000
2500
2000
1500
1000
500
0
CPM
No Ag
B6/Mouse
P1
P2
P3
Antigen
B
35000
30000
25000
20000
15000
10000
5000
0
CPM
No Ag
P2
P3
Antigen
C
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
CPM
Antigen

FIG. 5A-5C

A

Vaccine A stimulated line



B

Vaccine B stimulated line

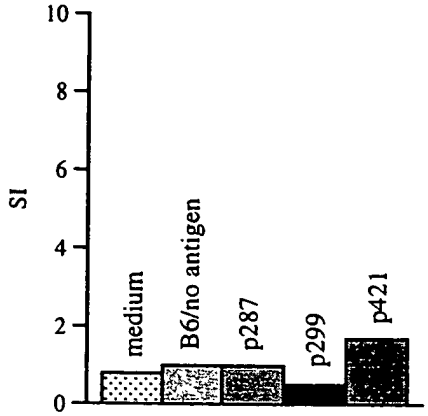


FIG. 6A and 6B

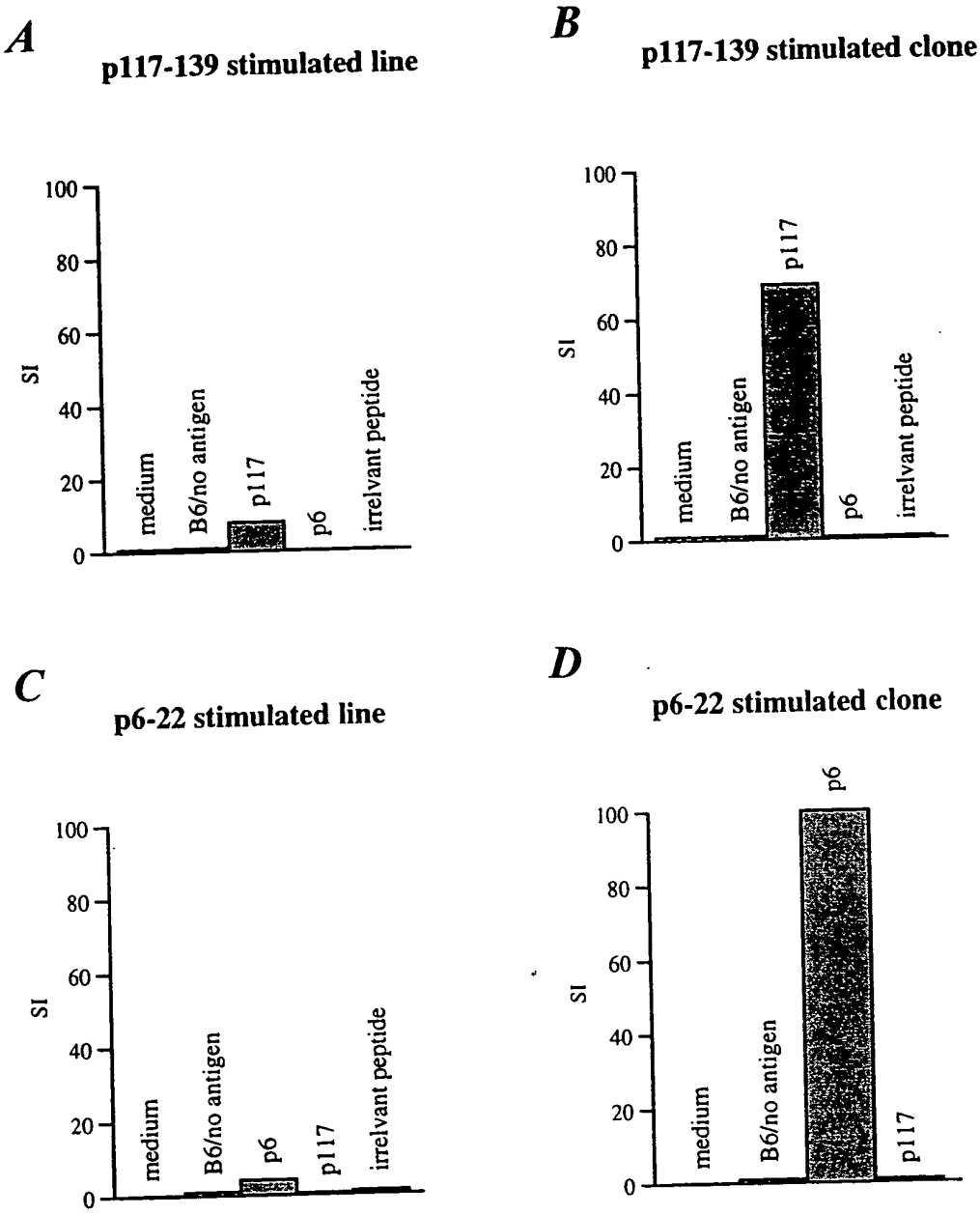

A
p117-139 stimulated line
SI
100
80
60
40
20
0
medium
B6/no antigen
p117
p6
irrelvant peptide
B
p117-139 stimulated clone
SI
100
80
60
40
20
0
medium
B6/no antigen
p117
p6
irrelvant peptide
C
p6-22 stimulated line
SI
100
80
60
40
20
0
medium
B6/no antigen
p6
p117
irrelvant peptide
D
p6-22 stimulated clone
SI
100
80
60
40
20
0
medium
B6/no antigen
p6
p117

FIG. 7A-7D

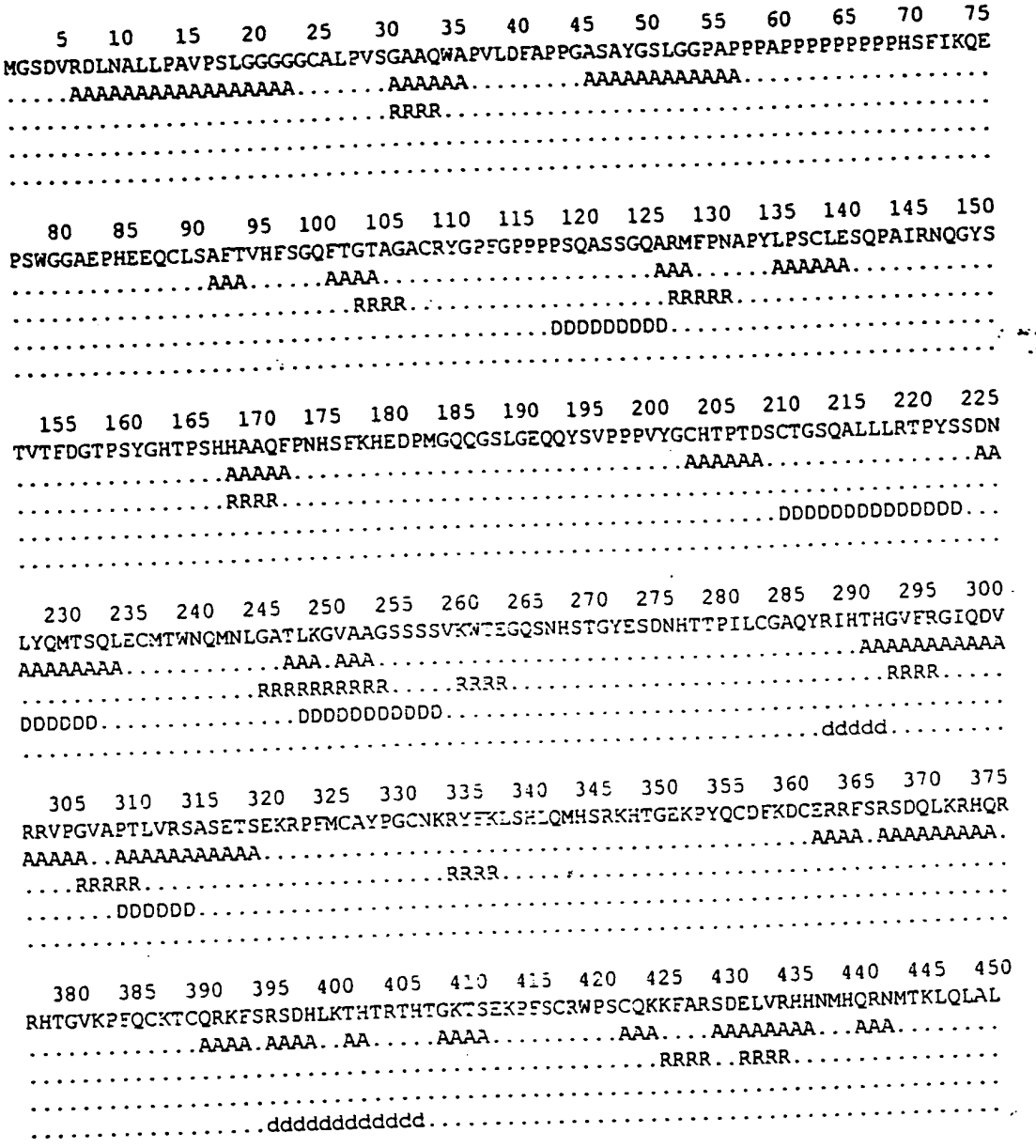

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
80 85 90 95 100 105 110 115 120 125 130 135 140 145 150
PSWGGAEPHEEQCLSAFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYS
155 160 165 170 175 180 185 190 195 200 205 210 215 220 225
TVTFDGTPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
230 235 240 245 250 255 260 265 270 275 280 285 290 295 300
LYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTEGQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDV
305 310 315 320 325 330 335 340 345 350 355 360 365 370 375
RRVPGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
380 385 390 395 400 405 410 415 420 425 430 435 440 445 450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWPSCQKKFARSDELVRHHNMHQRNMTKLQLAL

FIG. 8A

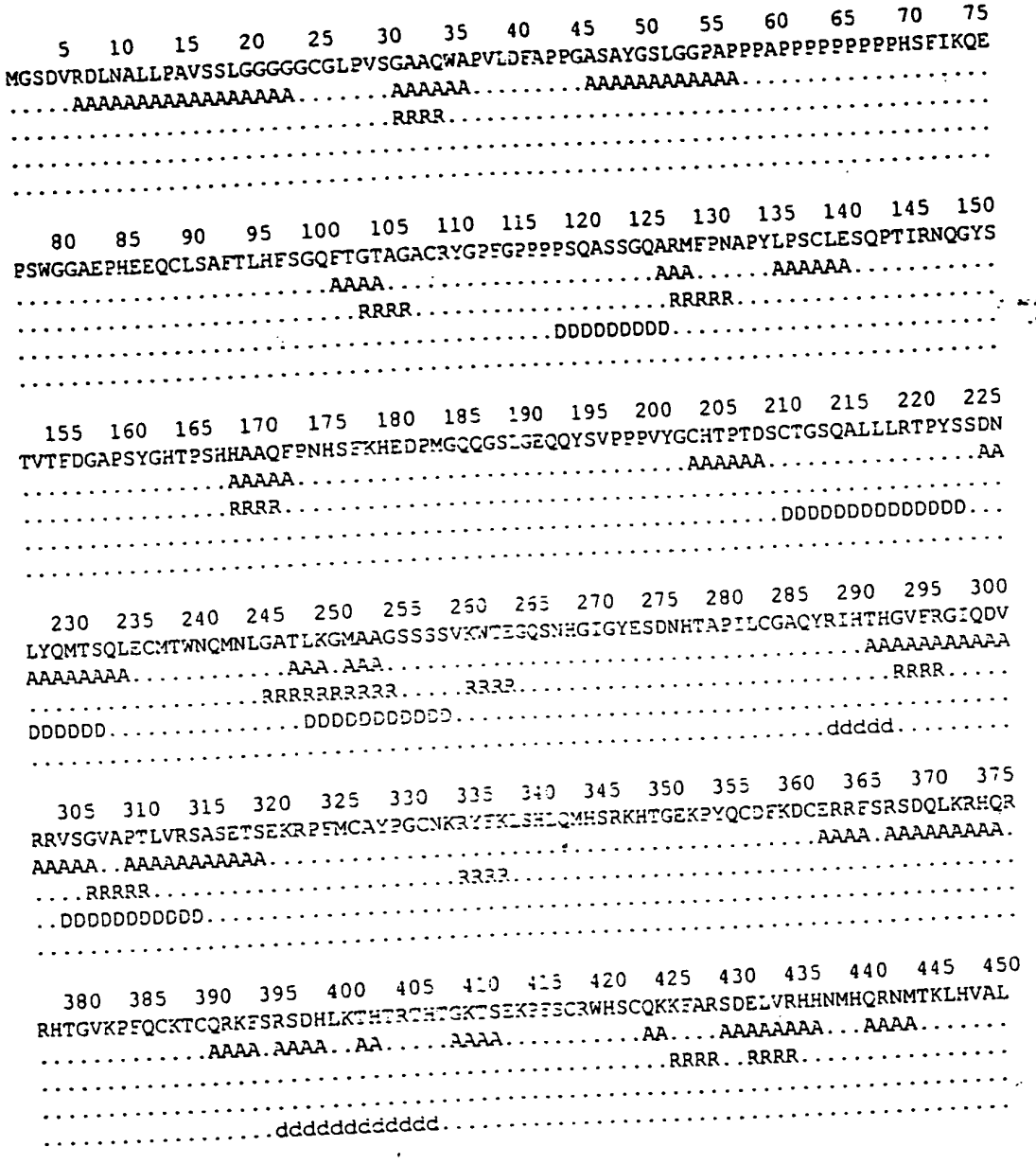
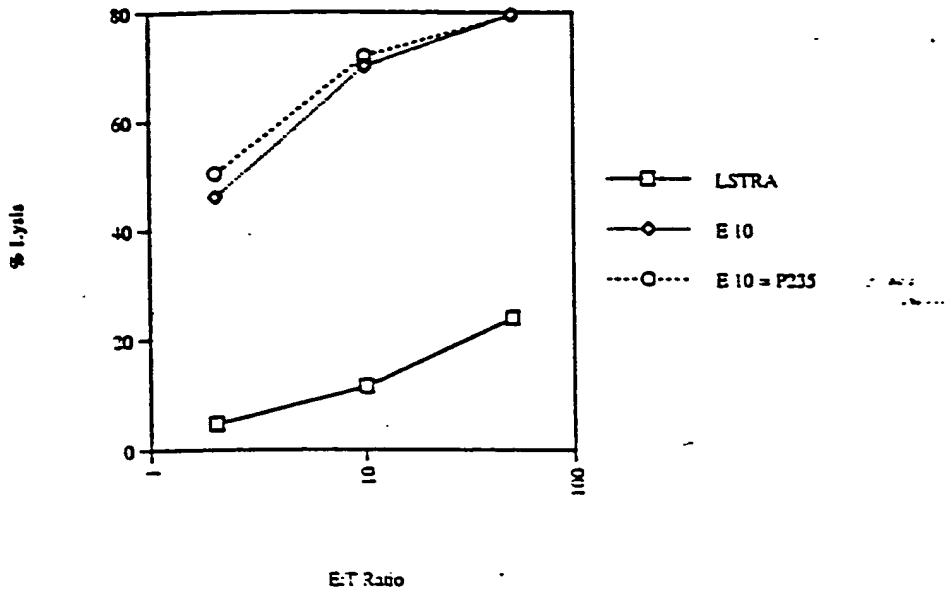


FIG. 8B

A



B

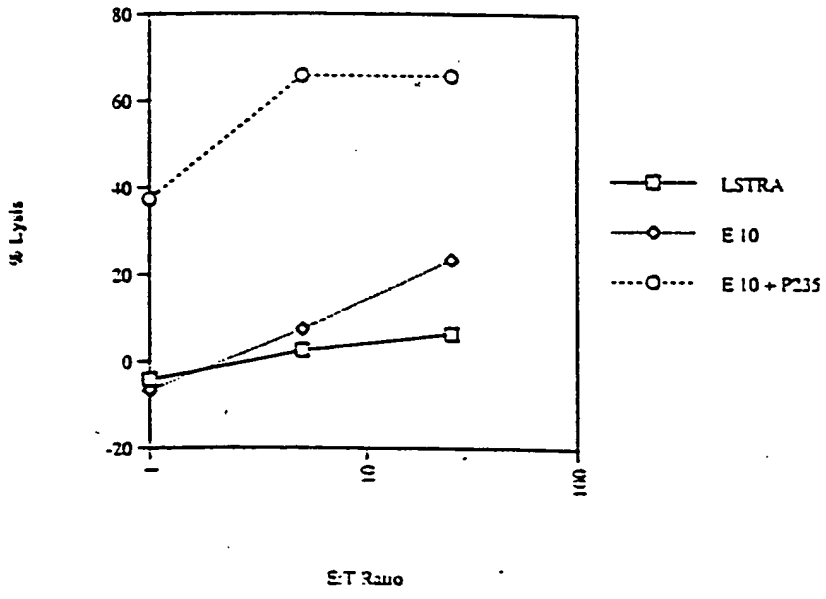


FIG. 9A and 9B

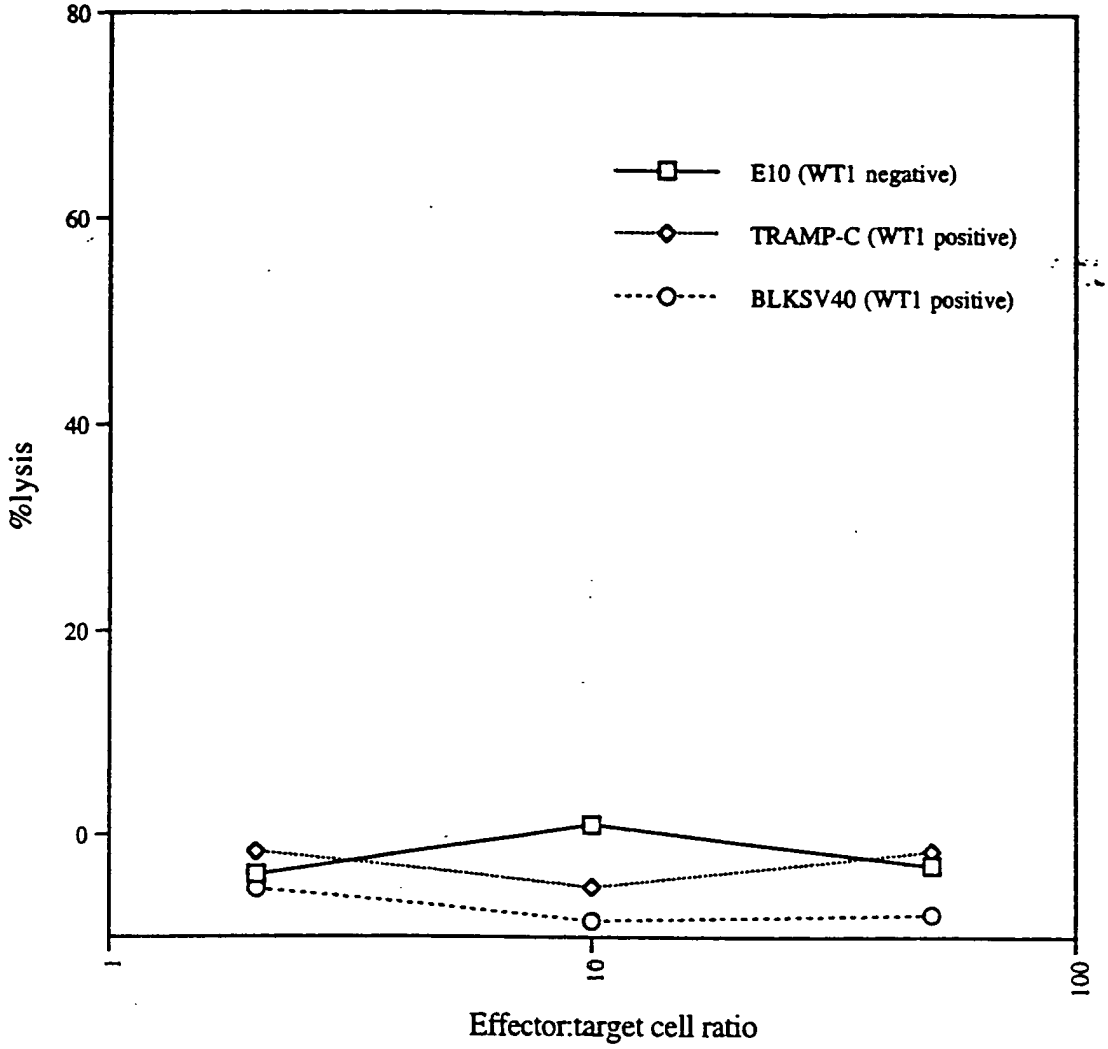

80
60
40
20
0
%lysis
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLKSV40 (WT1 positive)
1
10
100
Effector:target cell ratio

FIG. 10A

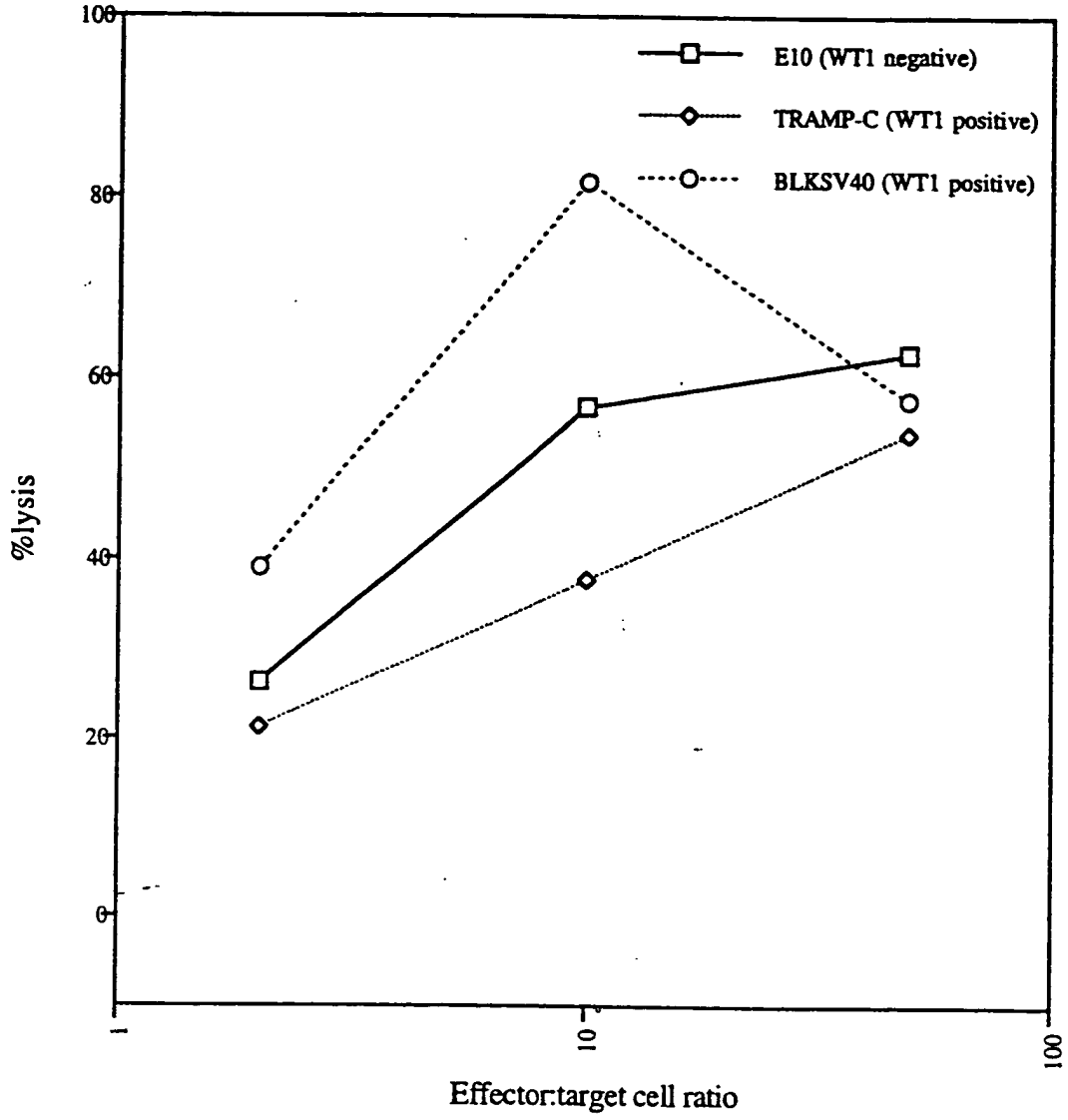

E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLKSV40 (WT1 positive)
%lysis
100
80
60
40
20
0
1
10
100
Effector:target cell ratio

FIG. 10B

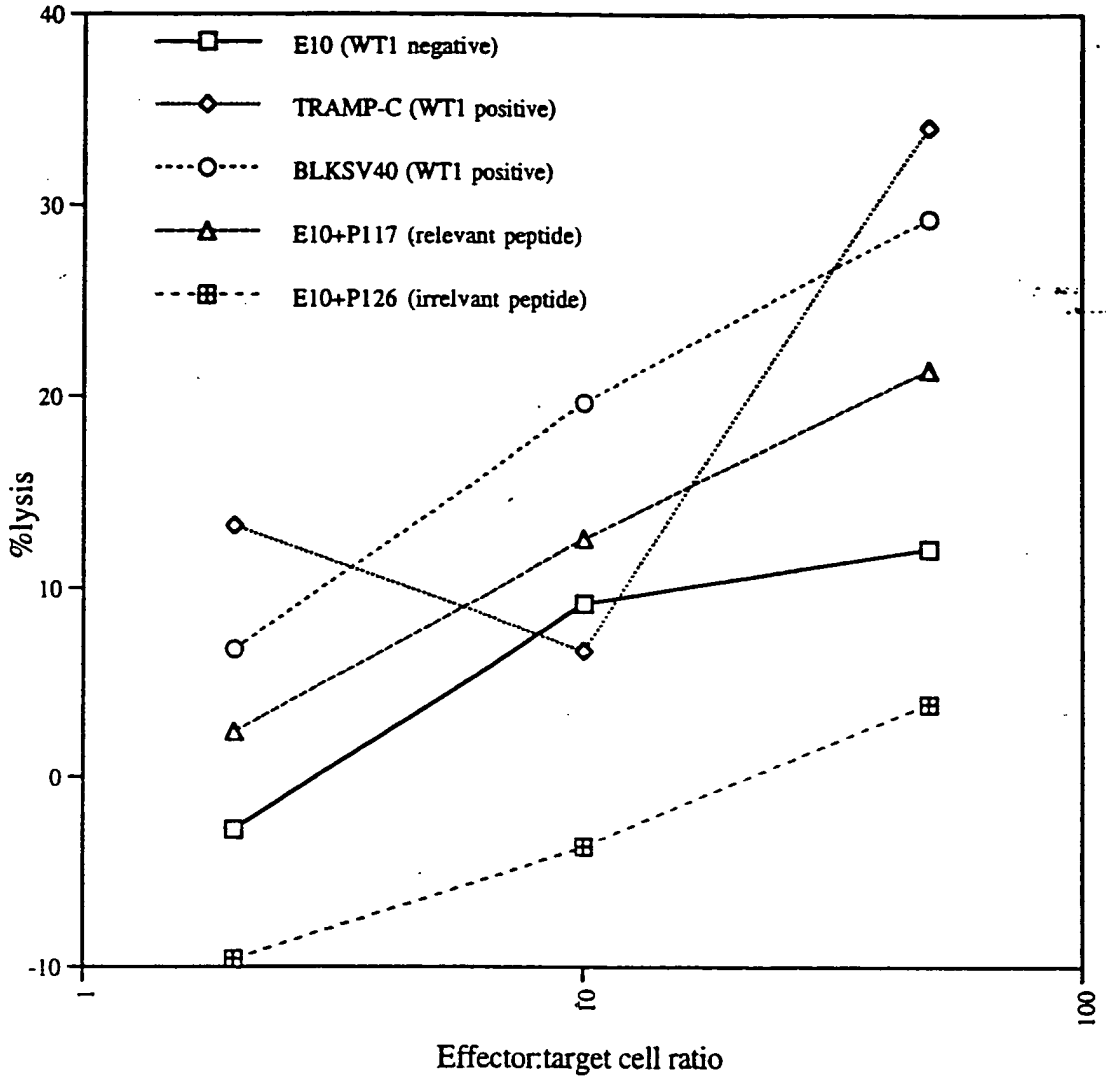

E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLKSV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelvant peptide)
%lysis
40
30
20
10
0
-10
1
10
100
Effector:target cell ratio

FIG. 10C

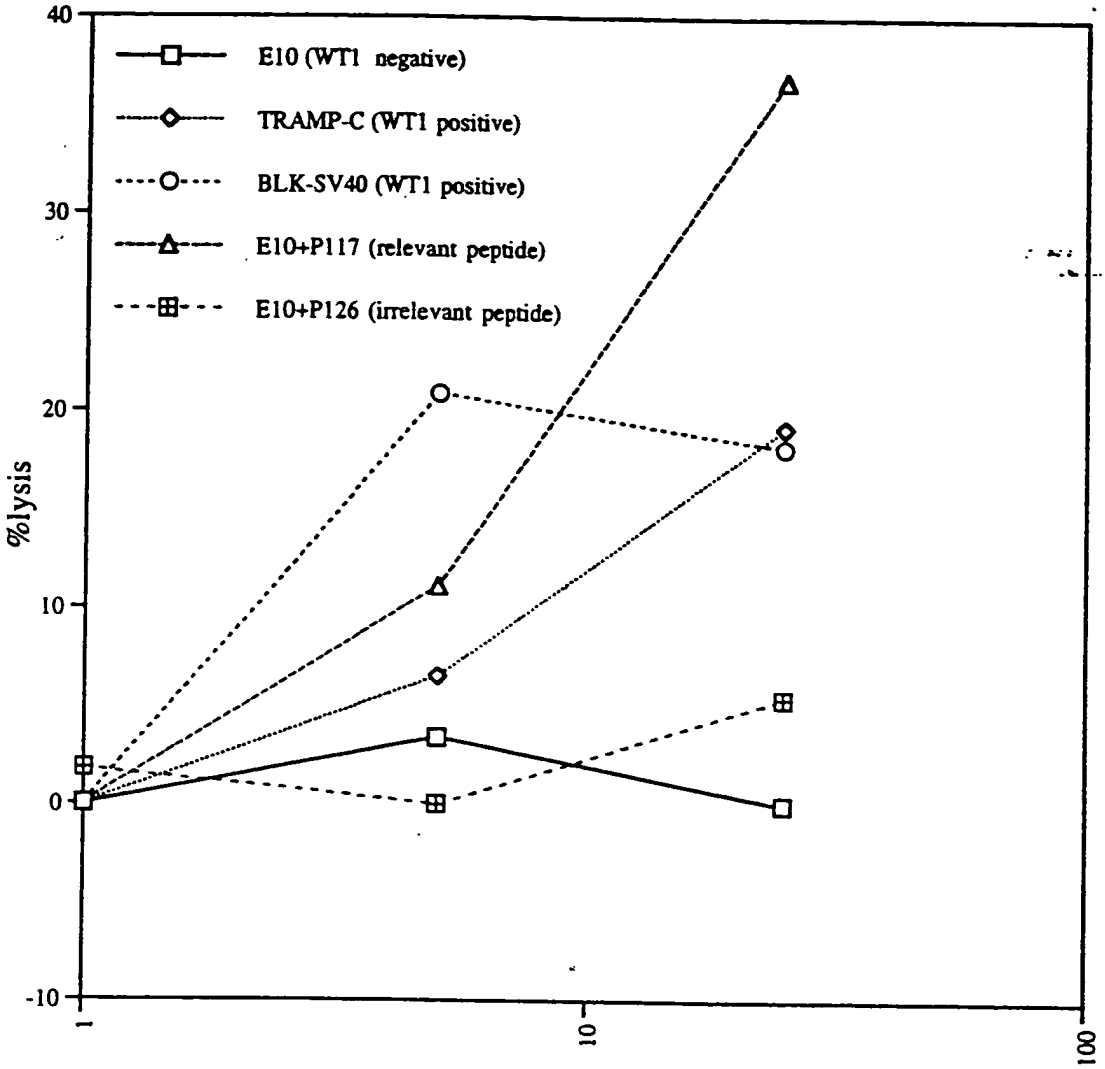

40
30
20
10
0
-10
%lysis
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelevant peptide)
1
10
100
Effector:target cell ratio

FIG. 10D

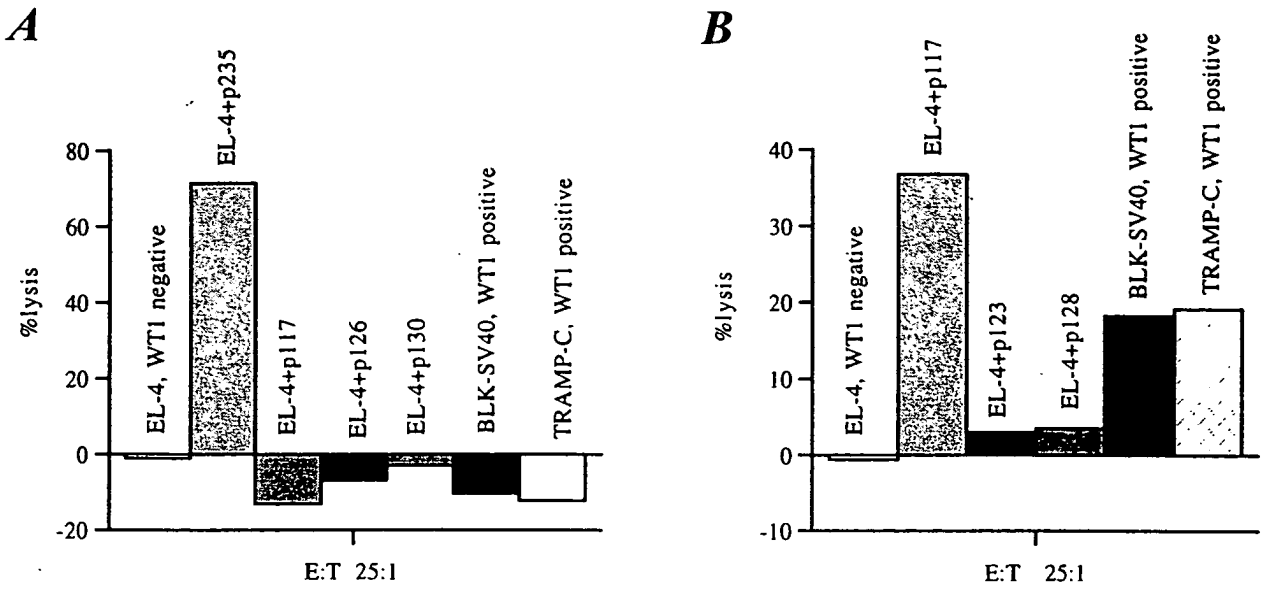


FIG. 11A and 11B

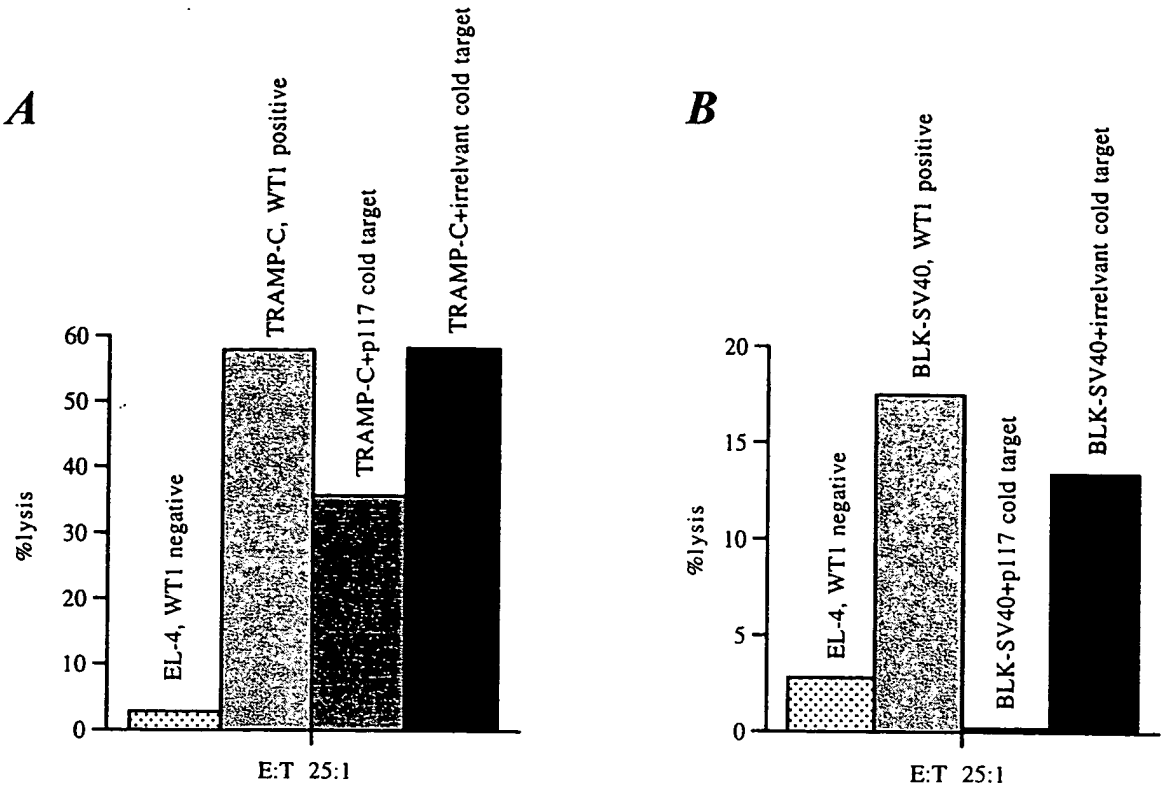


FIG. 12A and 12B

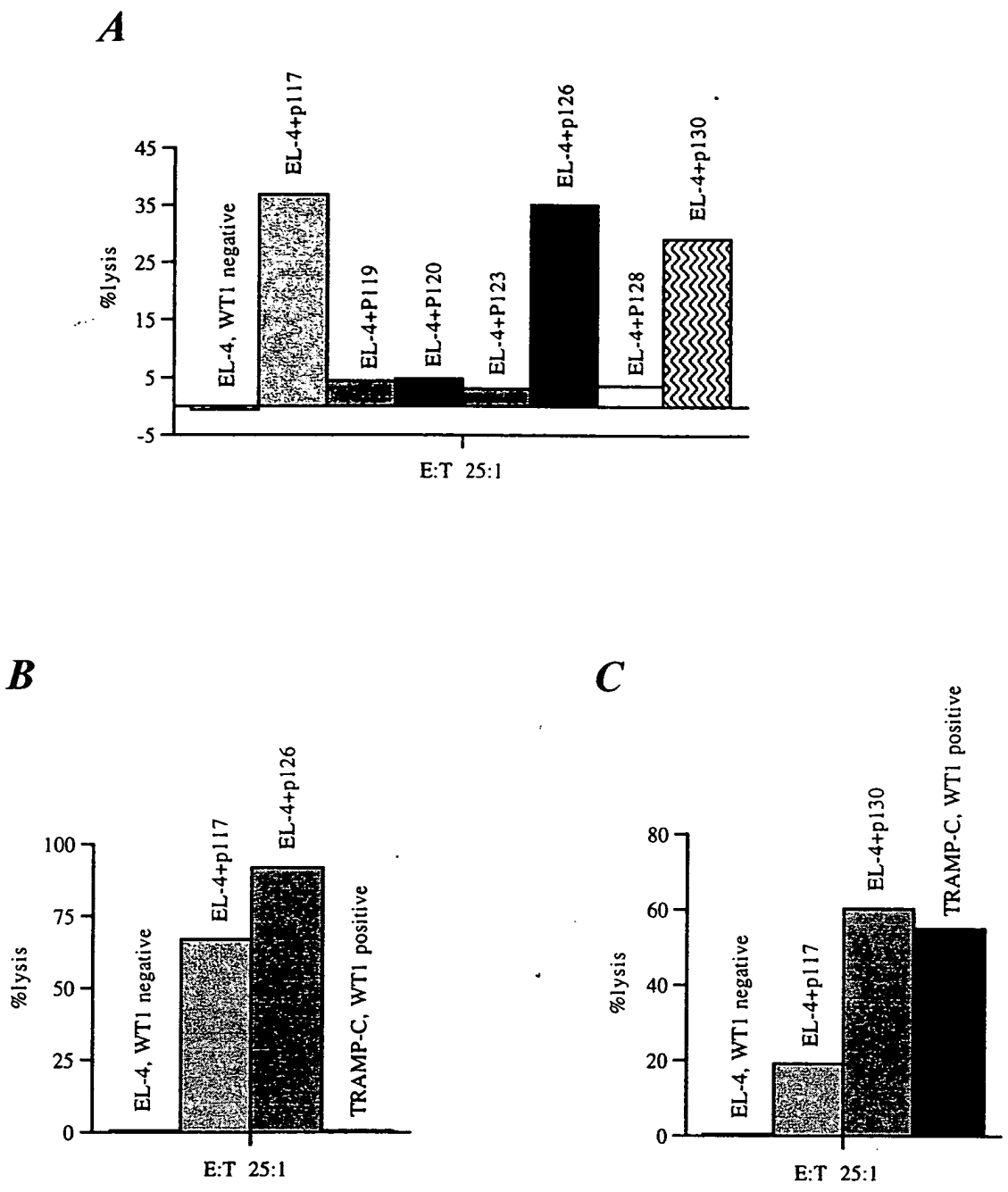

A
45
35
25
15
5
-5
%lysis
EL-4, WT1 negative
EL-4+p117
EL-4+P119
EL-4+P120
EL-4+P123
EL-4+p126
EL-4+P128
EL-4+p130
E:T 25:1
B
100
75
50
25
0
%lysis
EL-4, WT1 negative
EL-4+p117
EL-4+p126
TRAMP-C, WT1 positive
E:T 25:1
C
80
60
40
20
0
%lysis
EL-4, WT1 negative
EL-4+p117
EL-4+p130
TRAMP-C, WT1 positive
E:T 25:1

FIG. 13A-13C

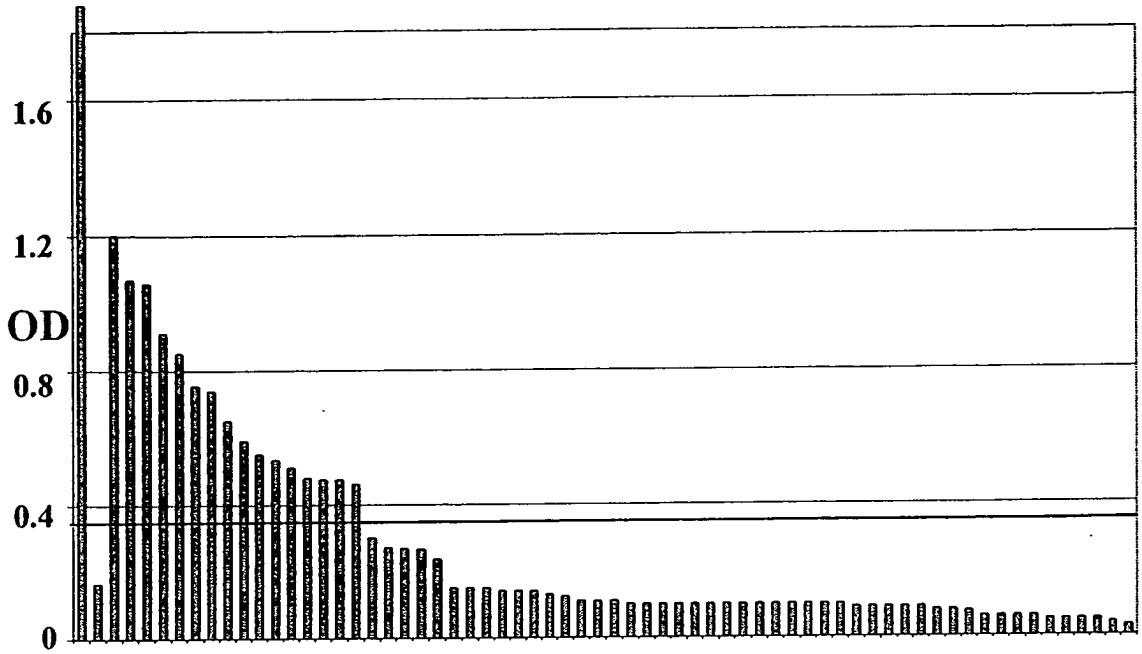


Fig. 14

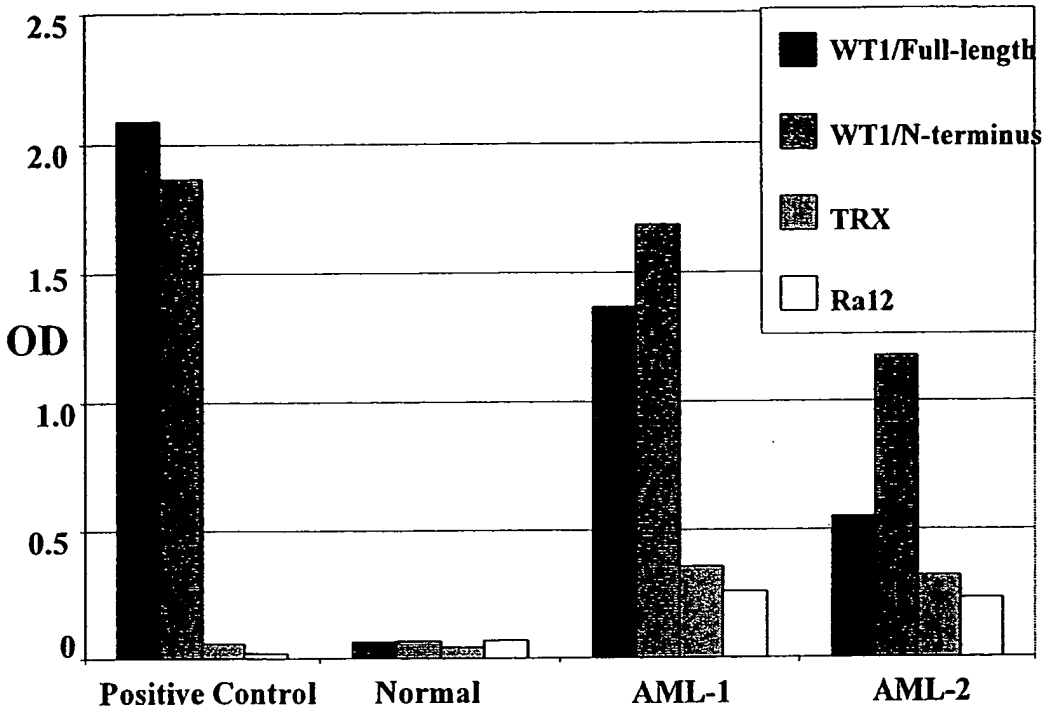

2.5
2.0
1.5
OD
1.0
0.5
0
WT1/Full-length
WT1/N-terminus
TRX
Ra12
Positive Control
Normal
AML-1
AML-2

Fig. 15

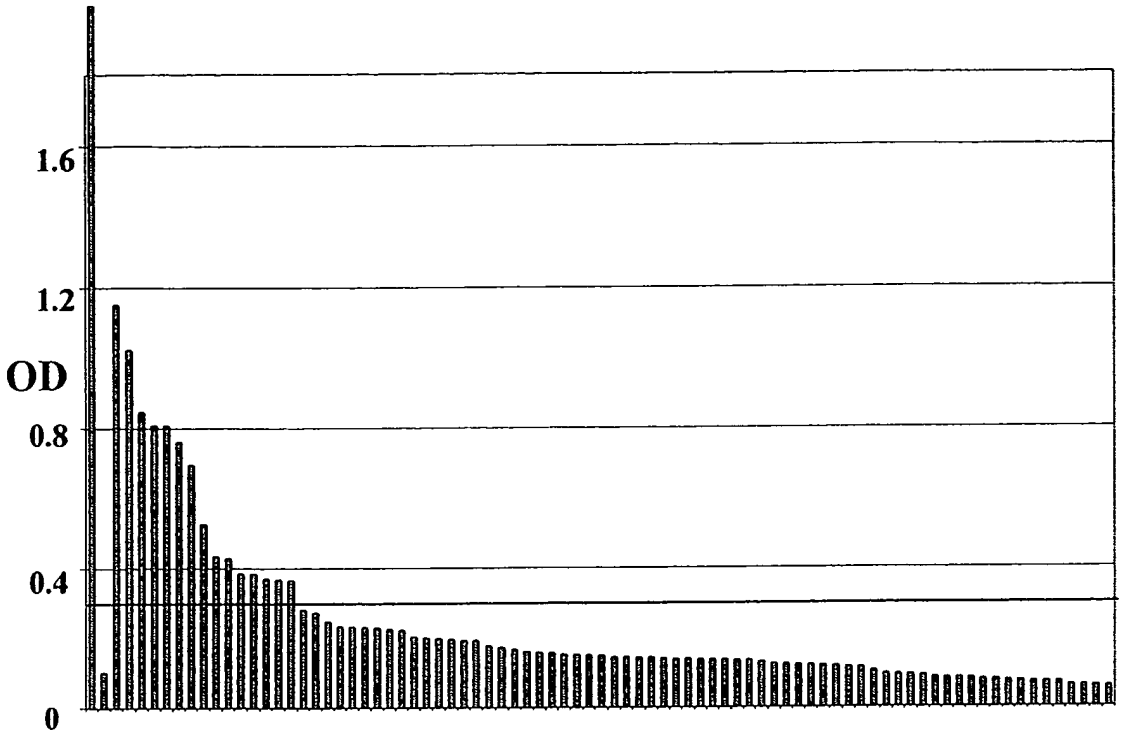


Fig. 16

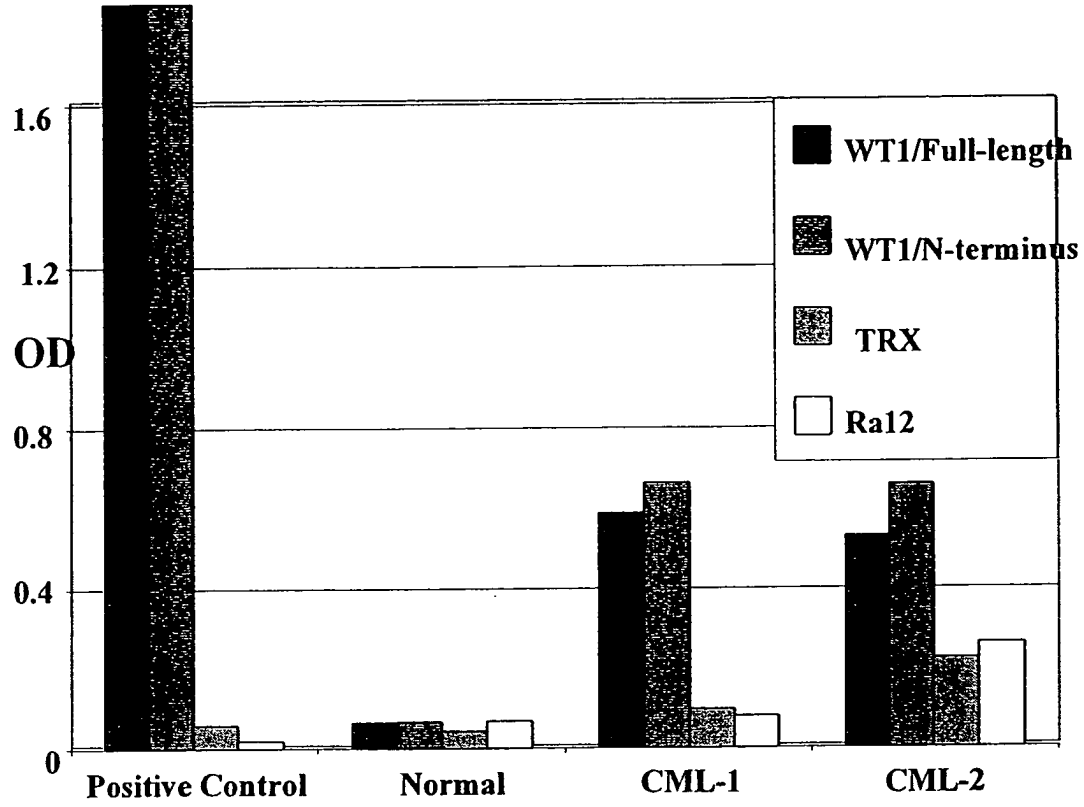

1.6
1.2
OD
0.8
0.4
0
Positive Control
Normal
CML-1
CML-2
WT1/Full-length
WT1/N-terminus
TRX
Ra12

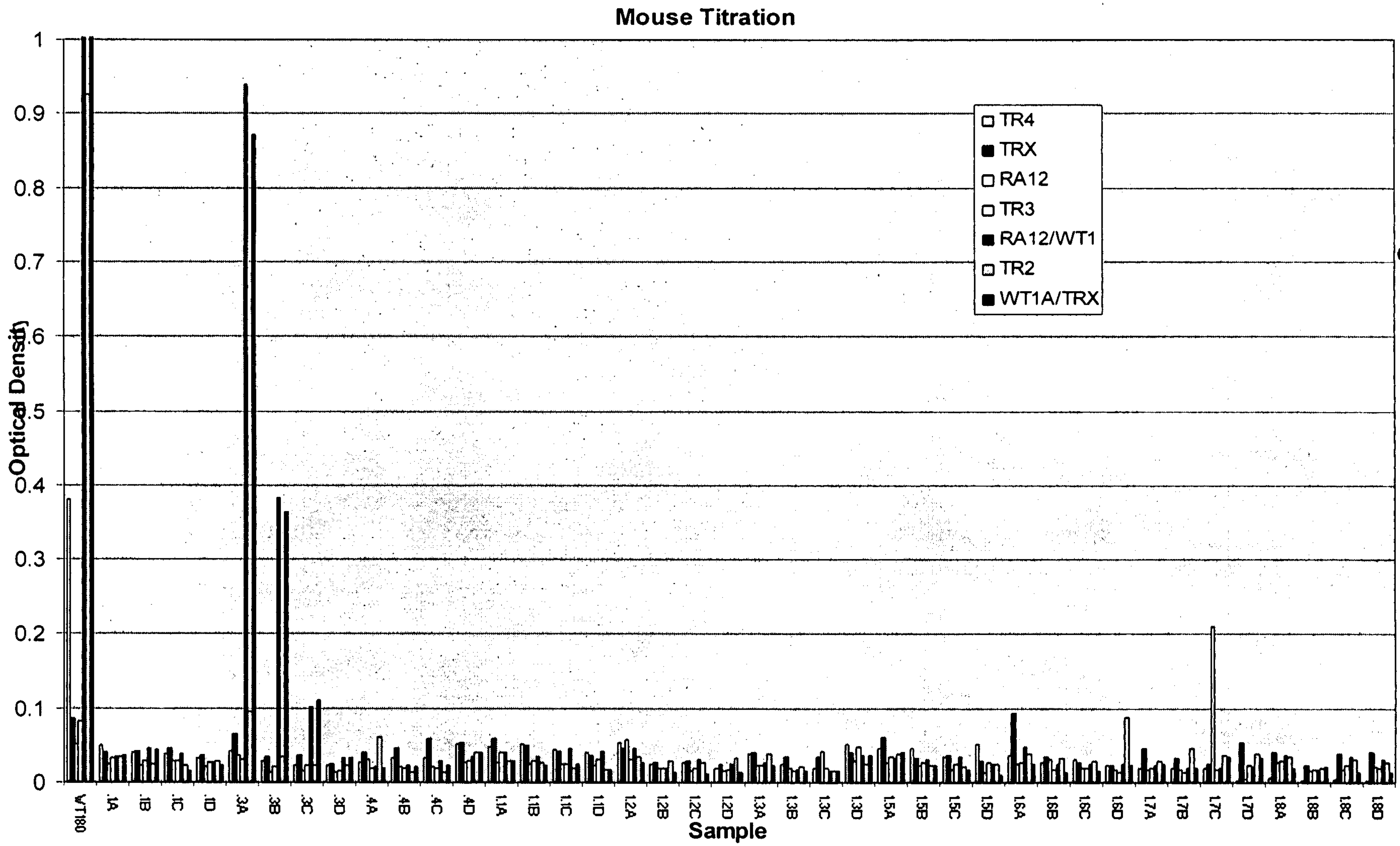
Fig. 17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

Name	Recombinant Protein	WT1 Amino Acid Position	Molecular Weight
WT1/full-length	Ra12-WT1 full length fusion protein	aa 1-449	85kDa
WT1/N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1/C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

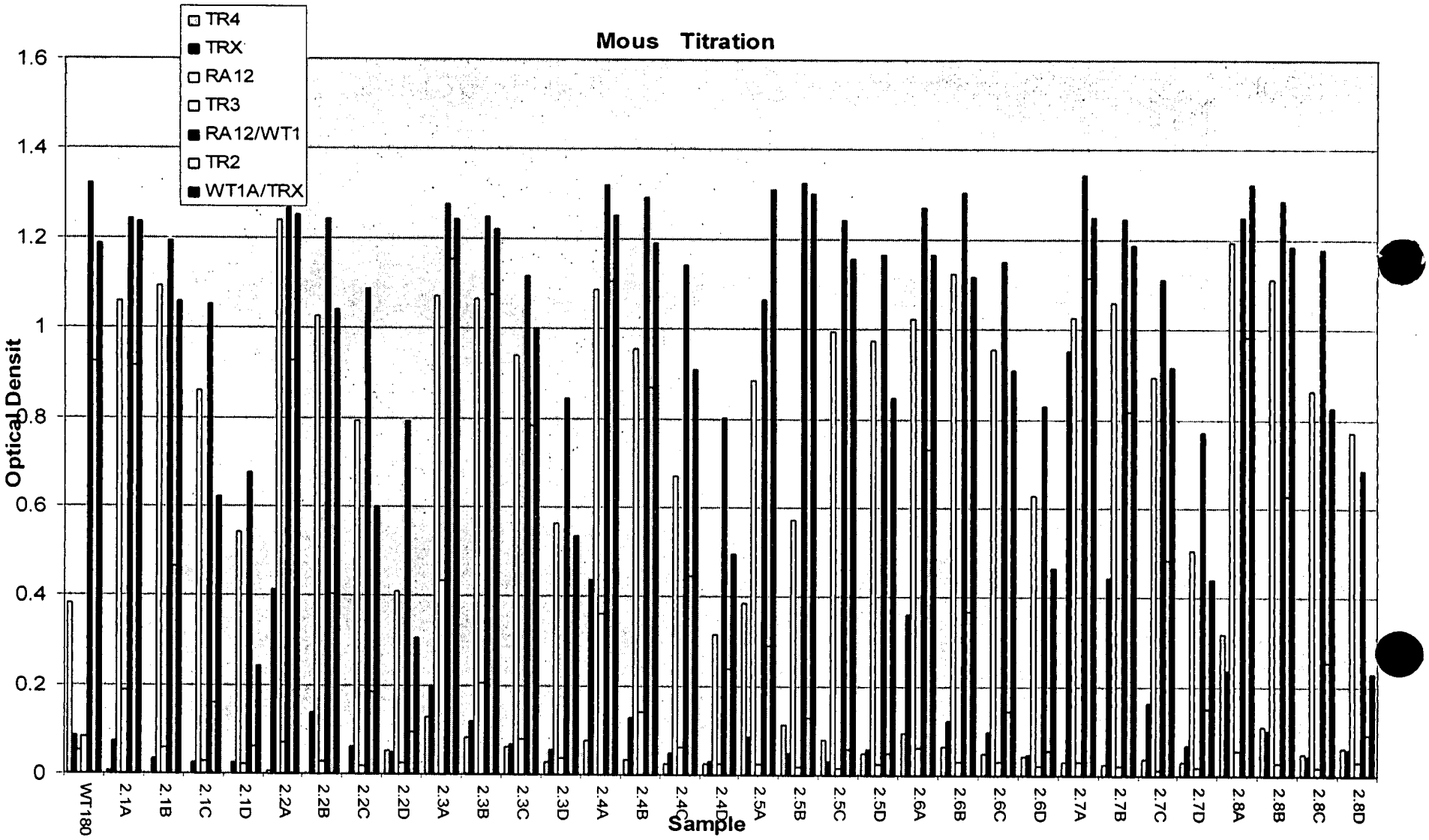
CID000622 Figure 1a Ab responses in group 0 and 1 (controls)



Control groups. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19A

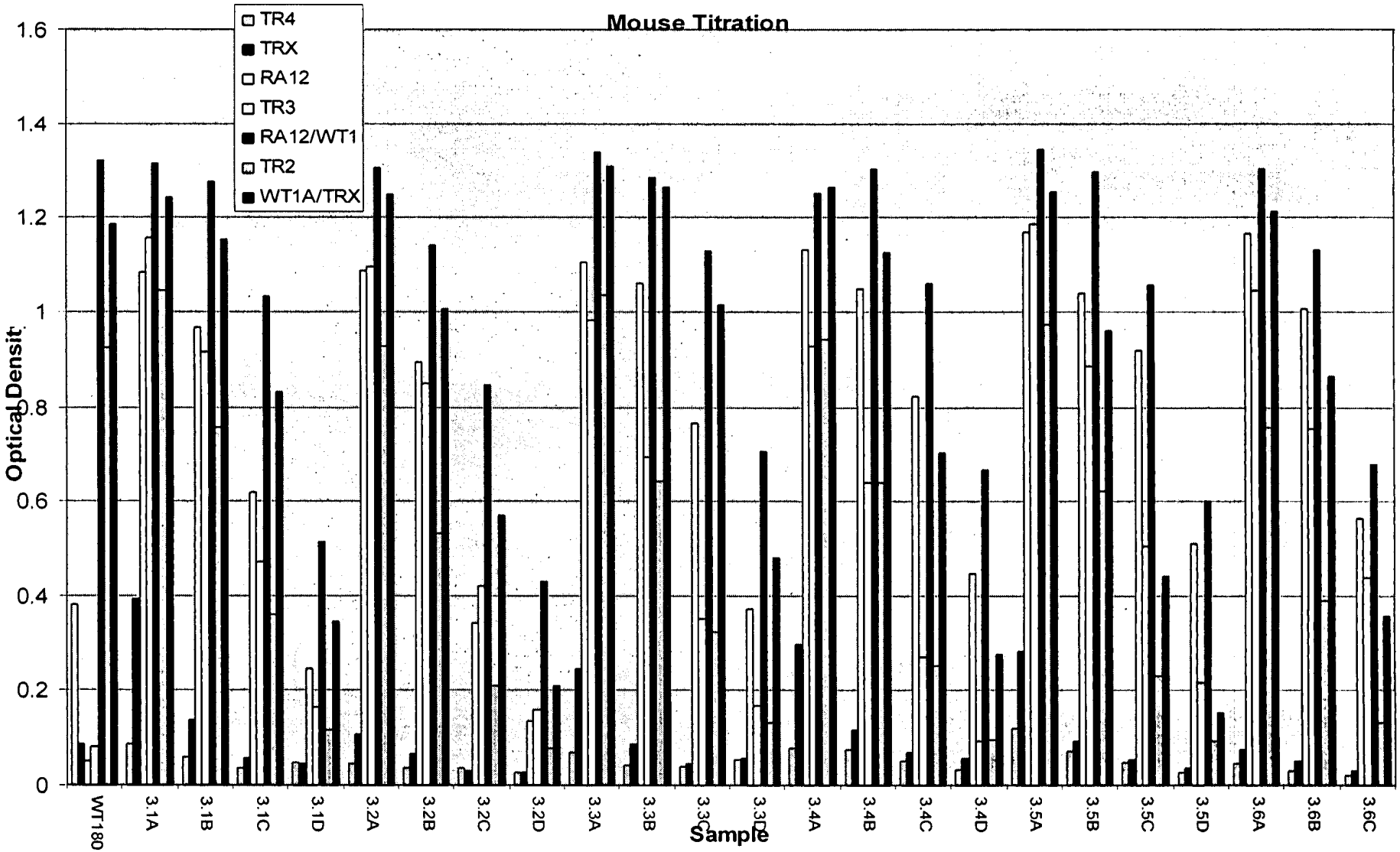
CID000622 Figure 1b. Ab responses in group 2 (25ug Ra12/WT1)



25ug Ra12/WT1+MPL-SE, A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19B

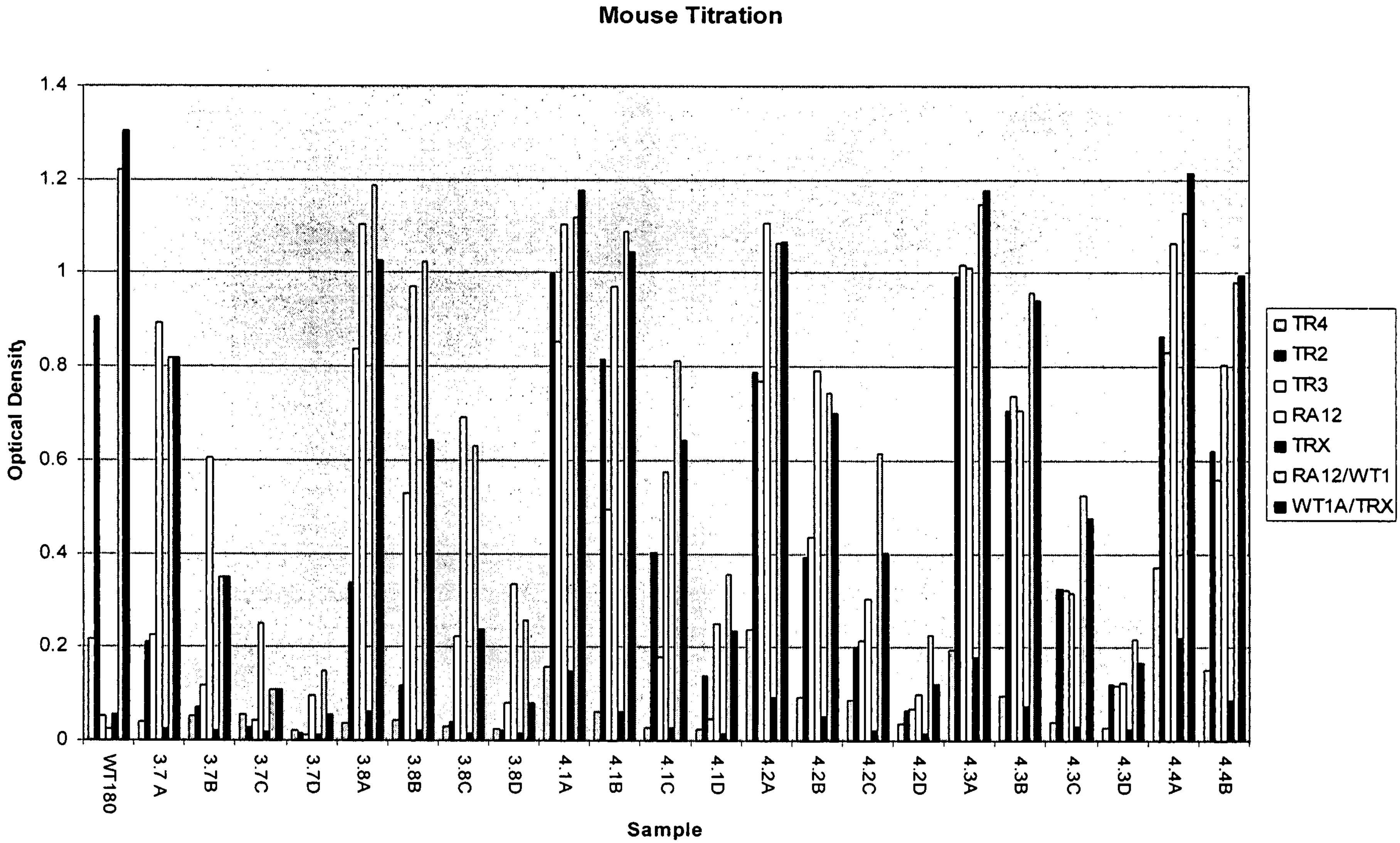
CID000622 Figure 1c. Ab responses in group 3 (100ug Ra12/WT1)



WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19C

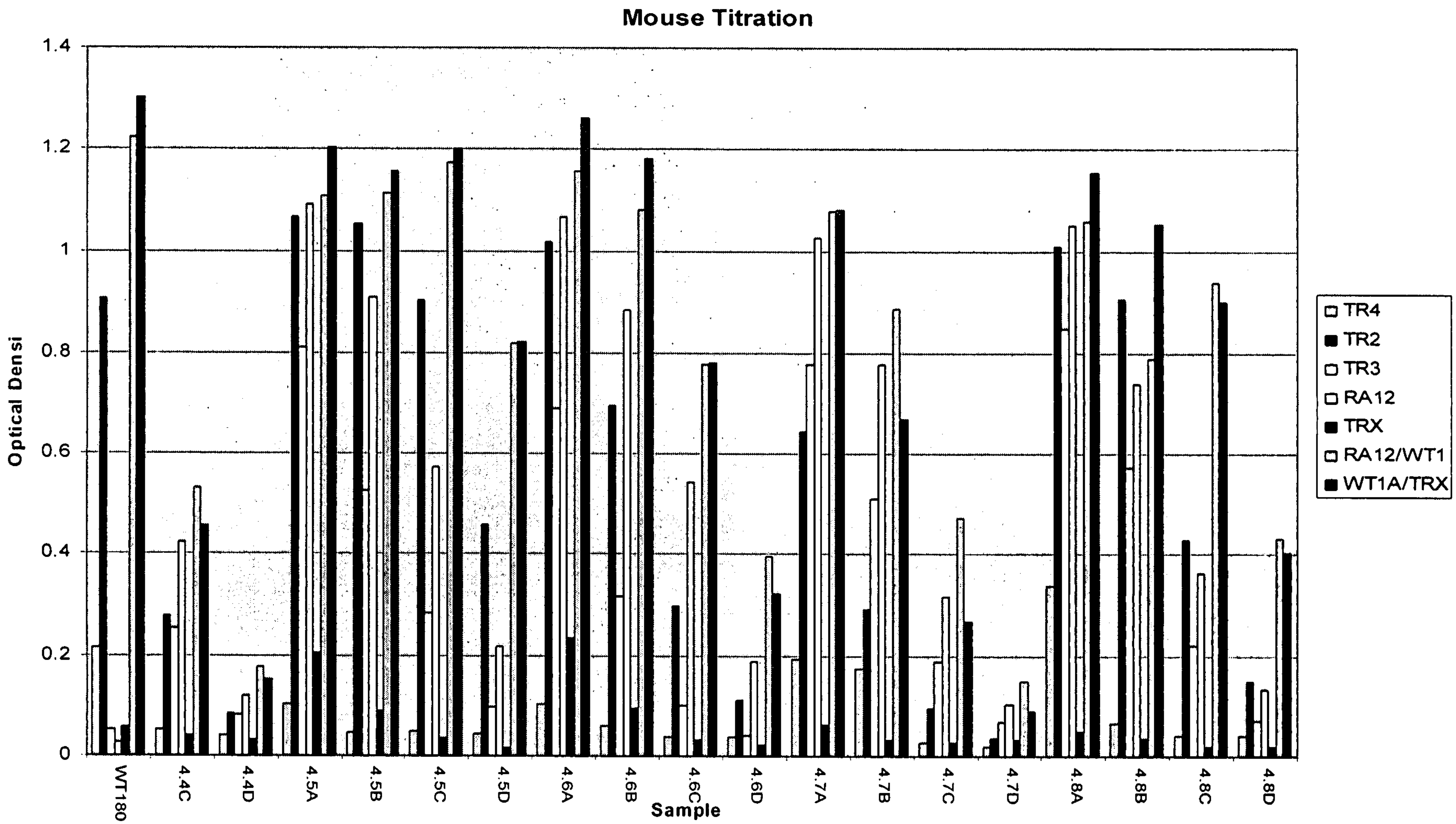
CID000622 Figure 1d. Ab responses in groups 3 and 4 (1000ug Ra12/WT1)



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19D

Figure 1e. Ab responses in group 4 (1000ug Ra12/WT1)



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19E

Figure 2a. Proliferative T-cell responses in WT1 protein immunized mice.
(Ra12WT1 dose titration, 3x in vivo, after 2IVS)

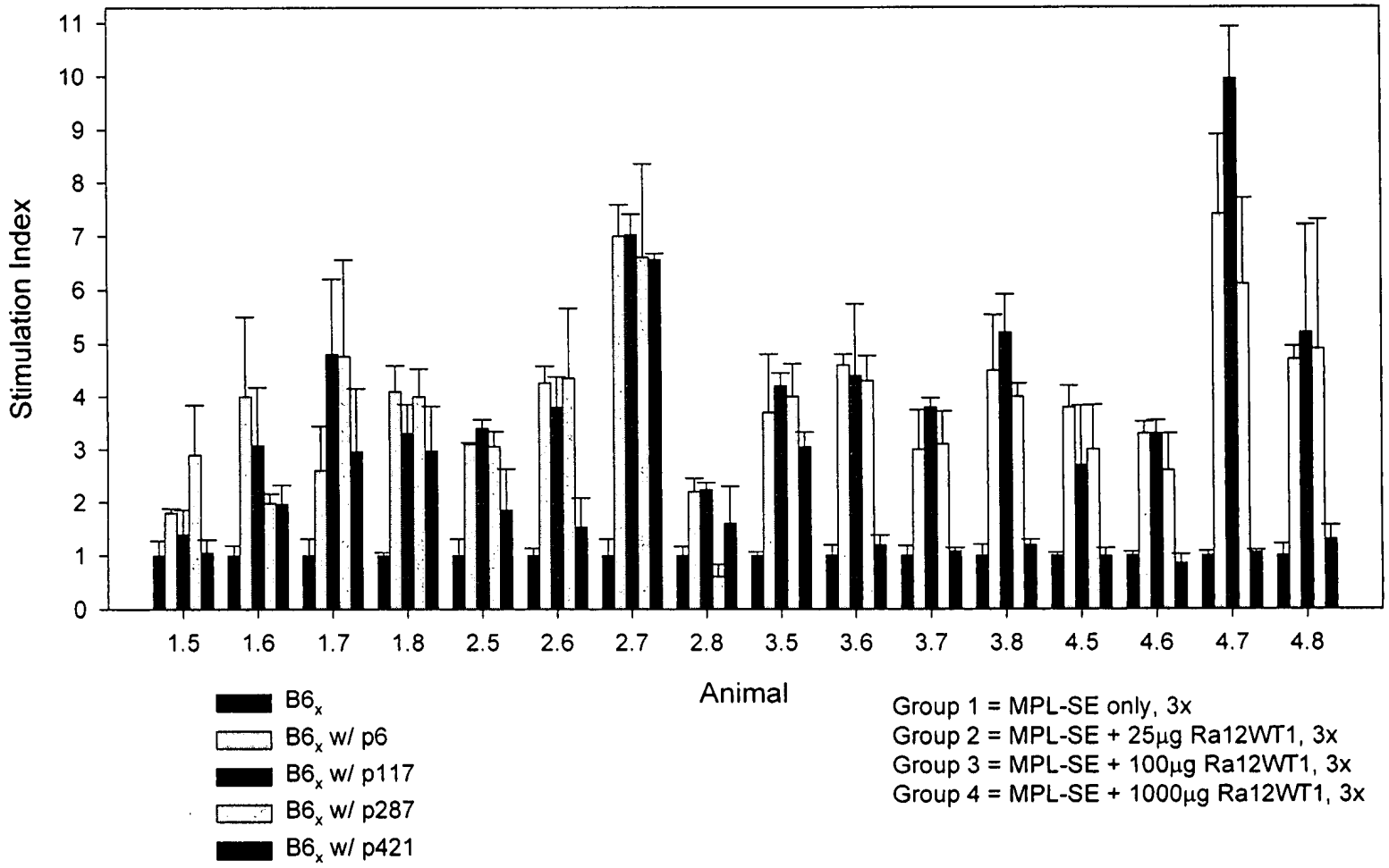


FIG. 20A

Figure 2b. Proliferative T-cell responses in WT1 protein immunized mice (Ra12WT1 dose titration, 6x in vivo, after 2IVS)

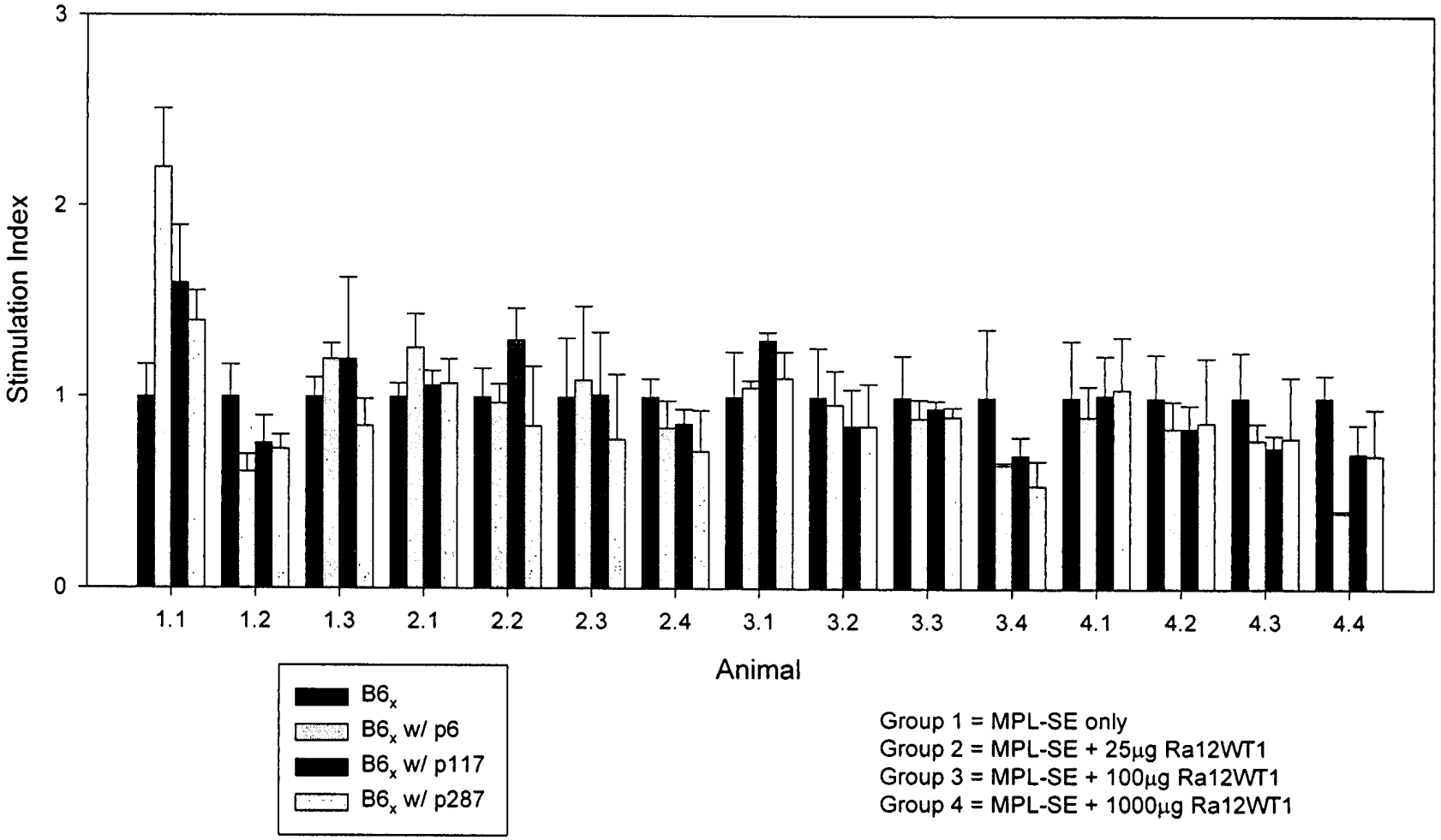
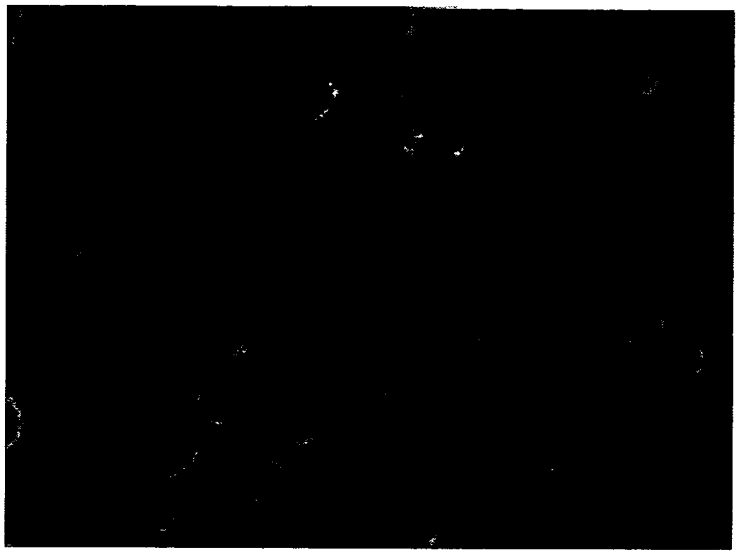


FIG. 20B

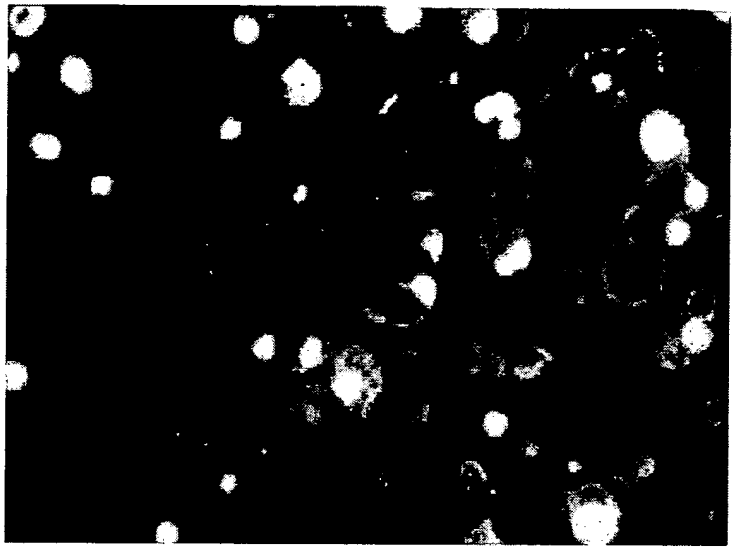
FIG. 21

Figure 1. WT1 expression in human DC following adeno WT1 and Vaccinia WT1 infection

Control (uninfected human DC



Adeno WT1 infected human DC



Vaccinia WT1 infected human DC

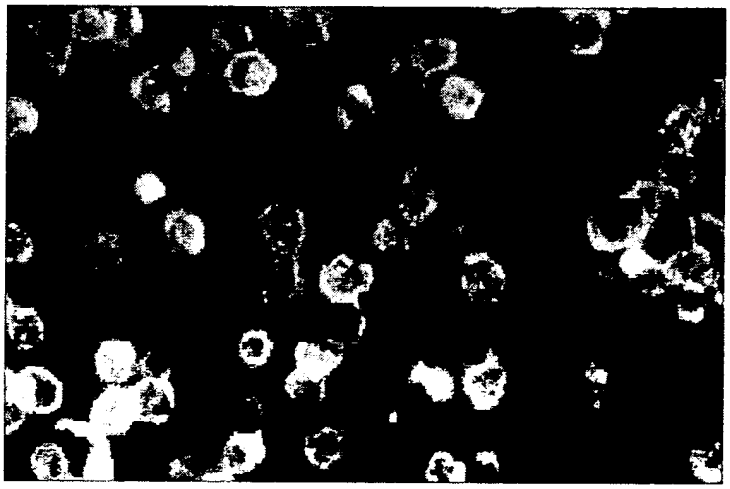
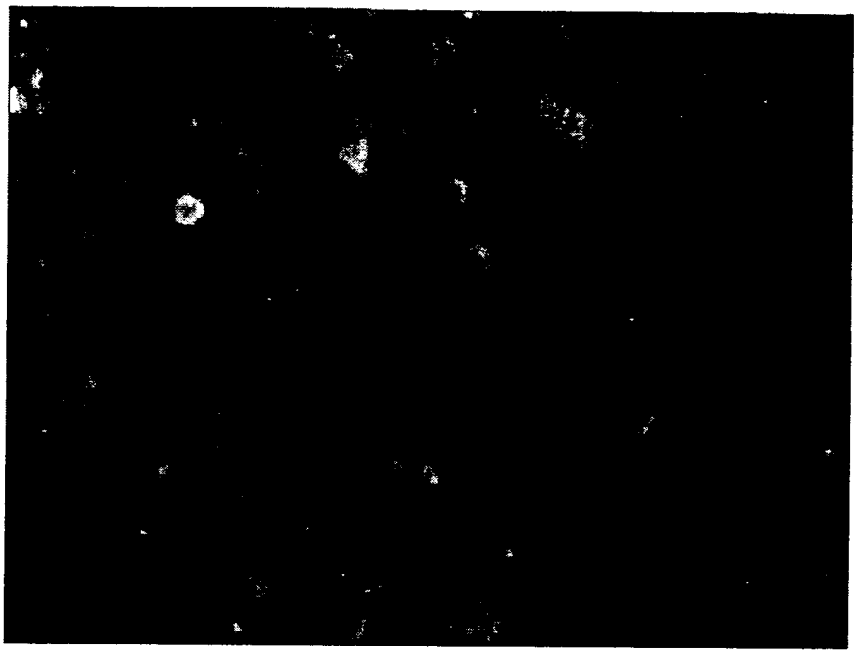


FIG. 22

Figure 2. WT1 can be expressed reproducible in human DC following adeno WT1 infection and is not induced by a control Adeno infection

Control (Adeno EGFP infected human DC)



Adeno WT1 infected human DC

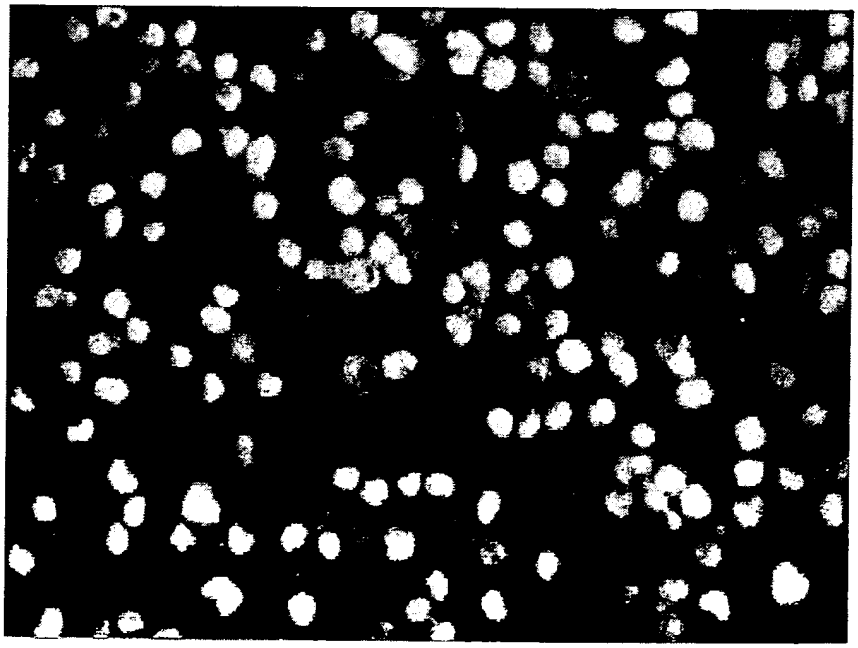


FIG. 23

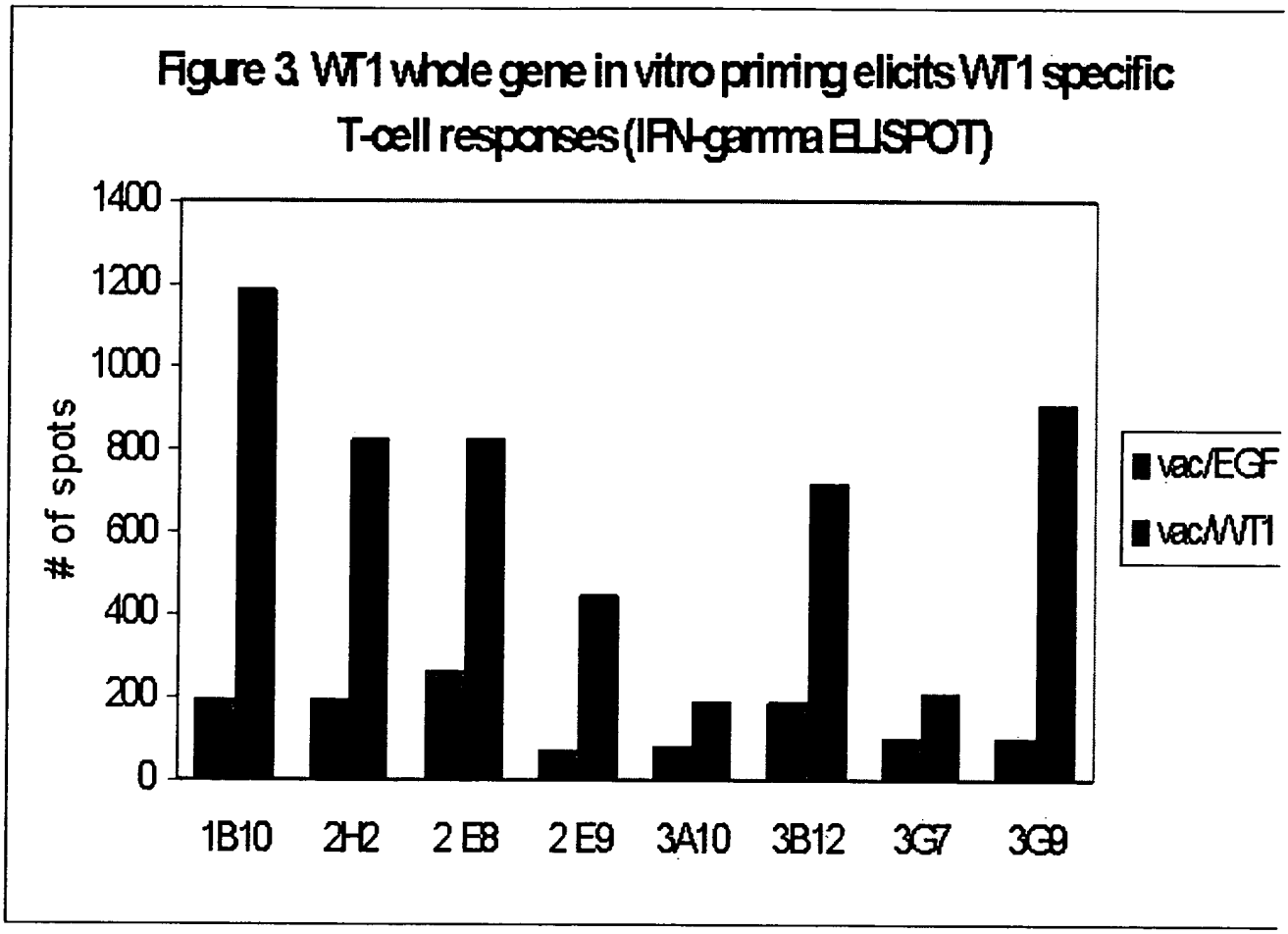


Figure 3. WT1 whole gene in vitro priming elicits WT1 specific T-cell responses (IFN-gamma ELISPOT)
# of spots
1400
1200
1000
800
600
400
200
0
1B10
2H2
2 E8
2 E9
3A10
3B12
3G7
3G9
vac/EGF
vac/WT1